# **FINAL Report**

# Bioassessment Monitoring of Acid Mine Drainage Impacts in Streams of the Leviathan Mine Watershed: Update for Spring-Fall 2009 Surveys

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## Introduction - Background

The pollution of streams by runoff from mining excavations can damage aquatic life long after mines have ceased operation. Acidic water, toxic metals, and contaminated sediments can combine to make affected sections of streams nearly uninhabitable. Restoration of water and habitat quality often requires a variety of remedies applied over many years. Recovery of natural biological communities can be used to evaluate the success of remediation programs and benthic or bottom-dwelling invertebrates are often used for the purpose of judging changes in ecological health. The studies reported here apply benthic invertebrate bioassessment for long-term monitoring of recovery in the Leviathan Creek watershed in the central Sierra Nevada.

Leviathan Mine is an abandoned open pit sulfur mine site located just north of Monitor Pass on Highway 89 in Alpine County, California. Covering an area of 650 acres (250 with visible mining disturbance), the mine last operated on a large scale in the 1950s and early 1960s, primarily for sulfur extraction. Acid mine drainage (AMD) from this site enters Leviathan Creek and Aspen Creek, flows 2.5 km from their confluence to become Bryant Creek where it joins with Mountaineer Creek, flowing a further 11 km where it enters the East Fork of the Carson River in Douglas County, Nevada. Acid drainage emanates from the following locations: the adit, the pit underdrain (PUD), the channel underdrain (CUD), the Delta Seep, and Aspen Seep. Together these discharges contribute acid drainage containing a mixture of dissolved and particulate toxic metals, and orange ferric hydroxide precipitates ("yellow-boy") to Leviathan Creek. In May of 2000 the U.S. Environmental Protection Agency (EPA) listed Leviathan Mine as a Superfund site to facilitate further site remediation and coordinate planning activities.

Discharge from the Adit and PUD is contained in collecting ponds. These ponds overflowed during late winter and spring snow-melt periods until 2000. The CUD and Delta Seep discharge directly to Leviathan Creek. Aspen Seep discharges to Aspen Creek. Active seasonal chemical treatment of AMD sources began in earnest in the autumn of 1999 and has continued since, with the result that the ponds have seldom overflowed since the spring of 1999. Pond water is seasonally treated through lime addition, settled to remove precipitates and then discharged to Leviathan Creek after

chemical testing. The CUD has also been intercepted and actively treated through lime addition, though only during the field season (approximately June or July through September or October depending on weather conditions). The Delta Seep was partly captured during the summers of 2003, 2004, 2007, 2008, 2009, and 2010. Treatment of CUD and Delta Seep is discontinued and discharges are returned to Leviathan Creek at the conclusion of each field season. Aspen seep has been treated year around in a bioreactor system since 1999. These actions have substantially reduced, but not eliminated the discharge of AMD to Leviathan Creek. During the period of 2004 and 2005, the most substantial changes in treatment regime were that in 2005 the CUD treatment period was shorter and capture of the Delta Seep was discontinued until 2007.

Bioassessment monitoring of aquatic invertebrates such as insects has been conducted since 1995 in streams of the Leviathan Mine watershed to provide an ecological evaluation of AMD effects on aquatic life and the progress of restoration. Benthic stream invertebrates are sensitive to chemical pollution and physical habitat disturbance and provide a useful indicator tool for assessment of biological integrity (Barbour et al. 1999, Rosenberg and Resh 1993). Aquatic macroinvertebrate bioassessment has been previously used to define the spatial extent of biological impacts in the Leviathan-Bryant Creek watershed in 1995, 1997, and 1998 through 2009, with most sampling also conducted in late spring and early fall of each year (June and September) and summarized in a series of report updates (Herbst 1995, 1997, 2000, 2002, 2004a, 2004b, 2007, 2009, and 2011). These data have established the ongoing changes in condition of the benthic invertebrate community along downstream AMD-affected sites and in reference streams, and document seasonal and year-to-year variations. The objective of this report is to provide an update for spring and fall 2009 bioassessment monitoring at sites in Leviathan and Bryant Creeks exposed to acid drainage discharges and an interpretation of ecological recovery. This continues development of a data set for evaluating the progression of improving conditions over time or relapses in health, and for use as indicators of the re-establishment of aquatic life to a natural state.

A group of 6 sample stations was surveyed in June of 2009 and 5 station in September, with Leviathan below mine (above Aspen) having dried by that time. The sample sites were located just below the mine on Aspen and Leviathan Creeks, on

Leviathan Creek just above its confluence with Mountaineer Creek, on Mountaineer Creek just above confluence with Leviathan, on Bryant Creek below the confluence formed by Leviathan and Mountaineer, and on Bryant Creek near the Stateline boundary (Figure 1). In addition to these sites, surveys have often included external control sites of similar in size to Leviathan and Mountaineer. In 2009 no control sites other than Mountaineer Creek were sampled. Control site sampling over the years of monitoring AMD-exposed sites are intended to frame background conditions of similar streams to represent the range of potential invertebrate communities that could be expected to occur in Leviathan and Bryant Creeks. The seasonal sampling times were selected to represent changing hydrologic conditions during spring run-off and fall base-flow, and phenological changes in the development of insect populations. Mountaineer Creek has served as the primary control site or reference for biomonitoring throughout the history of this survey program. To provide additional context for natural stream flow variation that may affect aquatic invertebrate populations, hydrographs through 2010 are shown for the East Fork Carson River (Figure 2), representing the larger watershed to which Leviathan, Mountaineer, and Bryant creeks are tributary, and for Bryant Creek below Mountaineer (Figure 3), to show local flow conditions in the Leviathan/Mountaineer/Bryant drainages.

#### Bioassessment Monitoring and Methods

The purpose of the monitoring program described here is to provide biological measures of ecological health using various attributes of the stream invertebrate community as indicators. These data will assist managers in delineating the area impacted by AMD, and establish a status condition for continued monitoring of the extent and progress of ecological recovery of stream habitat. Biological structure and function of aquatic ecosystems are not always obvious features of the environment, so practical field techniques are needed to assess the ecological health of streams. Aquatic insects and other invertebrates are central to the function of stream ecosystems, consuming organic matter (wood and leaf debris) and algae, and providing food to higher trophic levels (fish and riparian birds). These native organisms also have varying degrees of pollution tolerance and so may be used as indicators of water quality and habitat conditions. For example, distinctive shifts in the structure and function of the aquatic invertebrate community can often be detected above and below a pollution source. Such

use of the stream invertebrate fauna in evaluating stream ecosystem health is known as bioassessment. The technique relies on collections of the benthos (bottom-dwelling fauna) to evaluate the relative abundance of different taxa, feeding guilds, pollution indicators, and biodiversity, in order to develop a quantitative basis for measuring ecological attributes of the stream. Monitoring relative to reference sites (having little or no impact but similar physical setting), and/or over time within subject sites, then permits impact problems or recovery to be quantified (Rosenberg and Resh 1993). Previous studies of AMD impacts on stream communities have also utilized macroinvertebrate biomonitoring (e.g., Peckarsky and Cook 1981, Chadwick et al. 1986, Vinyard and Watts 1992, Clements 1994, Clements et al. 2000).

The approach taken for the set of long-term collections summarized here has been to use bioassessment sampling at a reference site (Mountaineer) for contrast to a core group of exposed sites located below the Leviathan Mine AMD source, and above and below the confluence with Mountaineer Creek. Data on the chemical properties of sediments and water from each sample site have also been collected to aid interpretation of biological patterns but are not included in this report. Past trends have shown gradual improvements in biological conditions progressing upstream toward the mine site contamination source area (Herbst 2009). Previous reports have examined patterns of biological impairment over the greater Leviathan Mine watershed including samples from streams above the mine, on the receiving waters of the East Fork Carson River above and below inflow from Bryant Creek, and on reference streams adjacent to the watershed (Herbst; series of reports 1995-2011). As with previous monitoring, sampling was conducted in late spring (May 18-19) and near early fall (September 14-15), within the index periods established for this study (late May or early June, and late September).

Bioassessment sampling was conducted by collecting benthic invertebrates from riffle habitats in shallow stream sections within established survey reaches. Riffles are turbulent flows of water over rocky, shallow stream reaches and contain the greatest abundance and diversity of benthic stream fauna. Samples were taken by kicking and flushing organisms by hand from rocks for 20-30 seconds into a 250-micron mesh D-frame net held just downstream of the 25 x 25 cm sample area (width and depth of the net). Large wood or rock debris was washed and removed from the net and the sample

procedure repeated at 2 more locations across each riffle transect. This composite sample of 3 collections was then swirled in a bucket, pouring off lighter suspended material to separate mineral from biological fractions (elutriation), the mineral fraction remaining in buckets was inspected in shallow white trays, remaining invertebrates collected, and the sample preserved in 95% ethanol. Such a collection contains benthic invertebrates in proportion to their relative abundance within the riffle sample areas. Five replicates of these composite kick-samples were taken at each site (moving upstream in randomly located riffle transects) as an estimate of sample variability for statistical description and comparison. Field sampling was conducted by a crew from the EPA Region 9 office, trained by David Herbst, with field supervision by Ned Black of the USEPA. The invertebrates collected were identified to the lowest practical taxonomic level (usually genus, species, or species group except oligochaetes and ostracods). Samples were sorted in the lab, organisms identified and counted, and data entered onto an Excel spreadsheet for analysis. Some dense samples were subsampled using a rotating-drum splitter and others were counted in their entirety (counts per sample typically averaged between 250-500 organisms). Laboratory subsampling, processing, sorting and identifications were performed on the first of the five replicate samples at the Sierra Nevada Aquatic Research Laboratory, and on the remaining four samples by the Environmental Research and Development Center (ERDC) of the US Army Corps of Engineers in Vicksburg, Mississippi. Staff of ERDC was trained by David Herbst so that all laboratory procedures were consistent with those used for previous samples.

Reference collections of all taxa have been established both at SNARL and ERDC to facilitate accurate identifications and for voucher archival. This provides a resource for comparing and verifying any taxa identified (preserved specimens and photos). For more details on methods and QA/QC procedures followed for these studies, see: http://www.waterboards.ca.gov/lahontan/water\_issues/projects/quality\_assurance\_project\_plan/index.shtml

Data were analyzed using descriptive statistics and graphical contrasts among sites and by season and time. The primary metrics used in interpreting community structure and biological integrity were based on measures of diversity, tolerance, density, and dominance. Mean taxa richness is a measure of overall taxonomic diversity for each

site and should increase with heterogeneity of habitat, spatial, and food resources. Mean EPT richness index is a measure of the diversity of generally sensitive insects belonging to the mayfly (Ephemeroptera), stonefly (Plecoptera) and caddisfly (Trichoptera) orders and will increase in clean, cold, well-oxygenated waters exposed to minimal chemical pollution or habitat alteration (calculated as the sum number of taxa in these groups in each sample). The biotic index is a composite measure of overall community tolerance to pollution and will increase (over a scale of 0-10) as water and habitat quality are degraded (it is calculated as the product of relative abundance and tolerance value for each taxon, summed over all taxa). The percentage of midges, particularly certain tolerant taxa, often increases within the sample under degraded conditions of water and habitat quality. Dominance is a measure of the relative abundance of the most common taxon and levels above 50% of the total community often indicate an imbalance or disturbance in food or habitat resources that permit one or a few species to dominate. Invertebrate density is often quite variable and less reliable as an indicator, but when pollution is severe, density of even tolerant taxa can be reduced as stream conditions become unsuitable for life.

## Results and Discussion

#### Quality Assurance Memorandum

From the 2009 sampling covering 11 surveys, over 21,000 individual organisms were counted and identified, comprised of 157 taxa. The first of the 5 replicate samples were sorted, identified and enumerated by D.B. Herbst at SNARL, while the others were processed at the ERDC lab (Mark Antwine and assistants). Confirmation of ERDC taxa (by DBH) was conducted by review of photographs taken with a microscope-adapted camera, viewed at an online photo archive. This procedure improves certainty in both identifications and counts, in making verifications and error corrections of ERDC samples, and in updating of the expanding reference collection photo archive. All samples contained a minimum count >250 organisms (except where severely impacted sites contained low densities), and average count was nearly 390 organisms per sample. Adjusted counts were made for some taxa that were mis-identified, but of 65 verification

checks, just 9 ERDC identifications were in error and were corrected. Error control is also ensured by comparing samples processed at SNARL with those at ERDC.

## Annual Trends by Site

The Leviathan watershed map of sites is shown in Figure 1, and hydrographs for the USGS gauges on the East Carson River and Bryant Creeks are shown in Figures 2 and 3, respectively. Summary of annual trends in primary indicator metrics are given in Figures 4-9). Note that for clarity of presentation only the means of the metrics (for the 5 sample replicates in each case) are shown in all the trend graphs, and each sample period is in sequence (some years without seasonal samples). The coefficient of variation of the principle metrics range from 5-10% for the biotic index, 15-25% for richness metrics, 25-50% for density and 20-30% for dominance. In previous surveys over this set of sites the most prominent pattern was of poor biological performance measures at the sites closest to the mine source area (Leviathan below mine, Leviathan above Mountaineer, and Aspen below mine). More recently there has been progressive upstream recovery, often approaching or exceeding the Mountaineer reference, occurring where and when AMD discharge has been controlled.

Mountaineer Creek. For most metrics, the trends observed in Mountaineer Creek have both been more stable and indicative of high quality biological conditions compared to trends observed in Leviathan Creek and Bryant Creek over the record of surveys. Metric values for 2009 were within the previously observed range. High values of the biotic index observed in spring 2008 indicate a greater proportion of tolerant invertebrates, and the typical pattern of lower spring densities than in the fall was reversed. Accompanying the change in 2008 was the occurrence of a dense growth of filamentous green algae in both spring and fall at Mountaineer Creek. While variation in metric values have occurred before, the coefficients of variation of the biotic index and richness metrics have been very low, at only 7 to 11 percent of the mean (the best metric performance for regional reference sites are in the range of 10-15%, Herbst and Silldorff 2006). The unusual high biotic index in spring was due to a greater abundance of midges, having higher tolerance values for pollution or habitat fouling by algae, and may use algae as

food or habitat cover (mostly *Eukiefferiella gracei*). Even though algae remained dense in fall, abundant midges did not persist. The appearance of dense algae at this site in 2008 is puzzling, but rapid growth of filamentous green algae is often associated with nutrient loading (e.g. nitrogen), so it may be useful to obtain nutrient chemistry along with metals analysis in future surveys. By the surveys of 2009, in the absence of algae, midges were no longer in abundance and biotic index was back in the typical range.

Flows and runoff timing may have important effects on stream invertebrates. Years 2000-2004 were regional drought conditions (Figure 2), and had the lowest winterspring cumulative flow during this period in 2001 (Figure 3), coinciding with a major drop in EPT taxa found in Mountaineer Creek (Figure 6). Low antecedent flows in winter-spring may not always result in declines in June EPT, as this did not occur in 2007 during similar low runoff. In any case, the diversity of EPT have always been higher in Mountaineer than at any AMD-affected site.

Seasonal increases in density from spring to fall at this reference site appear to recur with regularity (8 of 10 years with spring-fall data, but not 2008, Figure 8) suggesting that natural population demographics should follow this pattern, as recruitment, growth and development of many populations occur over this time. None of the AMD-exposed sites have exhibited this pattern with regularity.

Aspen Creek Below the Mine. Although gradual recovery at Aspen Creek below the mine, first noted in fall of 1999 as an improved (e.g., decreased) biotic index, had continued with the accrual of both mean number of total taxa and EPT taxa diversity through 2004 (Figures 4, 5, 6), richness measures declined from 2003 to 2006, but have rebounded starting in Fall 2006 and continued into 2009. Although the biotic index had risen over that time, it has now decreased to near that of other sites (Figure 7). Aspen Creek below the mine was first re-colonized by opportunistic taxa including the mayfly *Baetis* and the black fly *Simulium*, followed by the Nemourid stoneflies *Malenka* and *Zapada*. From low levels of abundance, the density of invertebrates had gradually increased at this site but was again lower in 2009 (Figure 5). Decreased dominance (Figure 6) and rising diversity suggests that a mixed community has become established at this site. The loss of richness and higher tolerance suggested over 2004-06, along with irregular fluctuations in diversity and dominance, may be at least in part attributable to

repeated livestock trampling of this small stream at the sampling locality. Collapsing banks, crushed and muddy ground cover, and erosion into this locale just above the fence-line, that were observed during 2004-2006, had not been noted in previous sampling. These livestock incursions to the site were stopped by 2008, and the site shows recovery in 2009. During this time there has been continued upstream treatment of stream flow through the Aspen Seep bioreactor. Despite these improvements, examining this site in the context of the eastern Sierra Index of Biological Integrity consistently scored this site as impaired in 2007-2008 (see page 16).

Leviathan Creek Below the Mine and above Mountaineer Creek. By 2003, the Leviathan Creek below mine site, closest to the mine, showed some early signs of recovery – increased taxa diversity, EPT numbers, reduced biotic index values, and lower levels of dominance by tolerant chironomids, though total density still remained low (Figures 4-9). In 2005-2006 these improvements were reversed, with losses in the diversity and density, and rising biotic index and dominance. Under what appeared to be a more effective and prolonged control of AMD discharges, the 2007-2008 levels of richness again showed an improving trend. In 2009 this site had flows only in spring and dried by fall. Conditions in spring showed no gains in diversity, but an increased biotic index and greater dominance as the pollution-tolerant midge Eukiefferiella claripennis became abundant (as seen previously). Densities remained very low. Further downstream, Leviathan at Mountaineer (above Mountaineer confluence), had also exhibited similar patterns of progressive recovery into 2004, evident in stabilization of the biotic index (as was noted in the initial recovery phase of Aspen Creek) and continued increase in diversity and density. The amount of yellow-boy deposition at this site had also appeared to be declining. The 2005 and 2006 surveys showed that recovery here too had been reversed evident in losses in diversity and density and increase in biotic index in 2005 after slight gains in 2004. Low levels of density of benthic invertebrates such as those observed at these Leviathan Creek sites shows how severely AMD can depress biological activity and biomass production. Low density remains a feature of Leviathan Creek. Just above the inflow of Mountaineer Creek, the lower Leviathan site showed that without dilution by uncontaminated flows, biological integrity had deteriorated during 2006. As of 2009 surveys, the trend shows continuing gains in diversity and stable densities

(Figures 6 and 8), with levels of the biotic index near the Mountaineer reference site (Figure 7, fewer tolerant taxa, and more sensitive taxa).

Bryant Creek. At sites below the mixing zone with clean flows from Mountaineer Creek, biological impairment has usually been less apparent than at the sites above Mountaineer and immediately below the mine. The Bryant below confluence sample station and Bryant middle station (also known as the Stateline site) appear to be the locations where the most extensive recovery has occurred and persisted in 2004-05 even while the Delta Seep releases were untreated. In 2006, the Bryant sites lost diversity (though maintained EPT), and had variable levels of density and dominance. At the same time, loss of sensitive taxa and/or gains in tolerant organisms were occurring below the confluence (increased biotic index, Figure 7), but not at the Stateline site downstream. While streambed substrates in these areas still showed traces and deposits of yellow-boy iron oxides, these sites were once densely covered by this precipitate when sampling began in 1995 and 1997. In the early stages of recolonization, these sites contained elevated numbers of some pollution-indicating taxa such as certain midges (e.g. Eukiefferiella claripennis grp., Corynoneura), empidids (Chelifera /Neoplasta), and mites (Sperchon), but have accumulated more total diversity and EPT taxa with time. The variable trends associated with these locations are typical of instable habitats in transitional phases of recovery, but absence of severe change in biological condition suggest sustained health and further rapid recovery are possible on upper Bryant Creek. In 2009, the Bryant Creek sites appear to be benefiting from reduced AMD discharge as they are within the range of the reference conditions at Mountaineer Creek.

Annual and seasonal trends for selected sites over the monitoring period 1997-2008 are presented in most of the Figures. The exclusion of spring 1995 surveys in all but Figure 7 is because the method used in 1995 involved collection from only one sample area for each of 3 replicates (resulting in low counts), while all other samples from 1997 on had sufficient counts or collected three combined samples for each of 5 replicates. The 1995 data will therefore underestimate measures of diversity and community composition, but not biotic index which is based on proportions. The mean taxa richness (Figure 4) shows that this measure of total diversity is typically in the range of 35 to 50 taxa at the Mountaineer reference site, and previously less than about 30 at the

AMD-exposed sites. Improving trends were apparent in 2003-2004 at all sites and again in 2009 after degrading some in 2005-06, and include the early signs of recovery at Leviathan Creek nearest the mine. As conditions improve in AMD-impaired streams, the community may shift from one of low-diversity, inhabited typically by a few species of very stress-tolerant organisms, to a transitional community of instable composition, dominated by "weedy" species (opportunistic colonizers such as the mayfly *Baetis*, and the black fly *Simulium*) that are often tolerant of metal contaminations, and a mix of more sensitive organisms. As improved water and habitat quality conditions persist, this instable phase is expected to be replaced by a more abundant, diverse and stable community of more equally-represented taxa, with varied food and habitat requirements, and regular seasonal patterns of population demography. Evidence of such patterns in community structure are present in control streams and during more complete effluent treatment periods, and should become more clear and predictable with continued trend monitoring during the ongoing remediation of AMD runoff.

Stages in progressive biological degradation or recovery related to AMD contamination may be discerned from changes in certain indicator organisms. About one-third of the total taxonomic diversity is found within one family - the Chironomidae or midges. Within this group are some of the best indicators or signal taxa for AMD pollution impact. Imbalance in community structure may first become apparent at moderately polluted sites (or those in initial stages of recovery) where *Baetis* alone may come to dominate >50% of all taxa. As severity of AMD exposure increases, *Baetis* abundance decreases while the relative abundance of midges increases. With further pollution the midge community itself comes to be dominated by Corynoneura and Eukiefferiella claripennis sp. group. Other taxa that appear in smaller numbers but are most prevalent at polluted sites include the empidid *Chelifera/Neoplasta*, the midges Pseudorthocladius, Pseudosmittia, the crane fly Molophilus, and the biting midge Monohelea. E. claripennis dominates where AMD pollution is chronic, and is present only in low numbers at unimpaired sites. This species group is a known indicator of degraded water quality conditions (Bode 1983), and in 2007 through 2009 was again abundant in Aspen and Leviathan Creeks below the mine in spring, becoming much less numerous in fall, possibly related to population phenology, or to deteriorating water

quality beyond the tolerance of even this species. Recovering communities are first recolonized by opportunistic taxa with rapid growth (*Baetis* and *Simulium*), and by a more diverse group of moderately sensitive taxa that are common and widely distributed (e.g. *Malenka*, *Ceratopsyche*, *Pagastia*, *Optioservus*). Dominance by these groups is then reduced as other more sensitive taxa can become established with further easing of AMD stress. [Examples of how combined metrics and overall community similarity can vary between sites and over time is shown in the graph examples on p. 16-17]

The decreased abundance and diversity of benthic macroinvertebrates in AMDaffected streams is a well-documented phenomenon (recently reviewed by Hogsden and Harding 2012), but there are few examples of how biological recovery proceeds. The use of biomonitoring as an indicator of ecological toxicity and mining-related pollution impacts and improvements has been substantiated through studies that show close correlation of bioassessment metrics with the standard bioassays using test organisms, and with dissolved metal contaminant concentrations (Schmidt et al. 2002, Griffith et al. 2004). Field studies on streams in the mining district of the upper Arkansas River in Colorado showed that within two years following water treatment that removed metals from contaminated inflows, EPT taxa increased and bioassessment metrics achieved upstream reference condition (Nelson and Roline 1996). Similar treatments on the Clark Fork in Montana required much longer periods for aquatic invertebrate recovery to occur (Chadwick et al. 1986), but were complicated by flows redistributing metal-contaminated sediments (Hornberger et al. 2009). Bioassessment monitoring of the Leviathan Creek watershed has also shown mixed results, with recovery occurring during periods of effluent control to the stream, and relapse to degraded conditions when AMD pollution has not been abated, or when unrelated disturbances such as livestock grazing incursions have occurred on Aspen Creek.

The algae and organic matter food resources of benthic invertebrates become reduced in streams exposed to AMD. Growth of most algae on stream bed surfaces is severely decreased under lower pH, elevated metal concentrations and when metal hydroxides such as yellow boy coat and cover substrata (Niyogi et al. 1999, Verb and Vis 2001). Microbial decomposition of leaf litter and wood that fall into streams is an integral trophic resource in forested watersheds, and the bacteria and fungi that mediate

this process are impaired by AMD (Niyogi et al. 2002, Schlief 2004). These results show that AMD may alter ecosystem processes of primary production and decomposition, changing food resource availability and distribution, forcing food webs into simpler and less productive pathways. These kinds of changes in organization of Leviathan stream communities can be examined in the functional feeding group structure between and among sites over time. This may contribute to a more complete understanding of AMD impact and recovery on stream function and productivity.

AMD poses multiple stresses on benthic invertebrate communities. Chemical stressors include a mix of toxic metals (e.g., As, Ni, Al). Given the physical effect of chemical precipitates that may cover surfaces, this may prevent inhabitation of substrata. It may be possible to account for the presence and extent of these coatings in the iron oxide content given in sediment quality samples (such as those collected at Leviathan by N.Black of USEPA). This then would permit separating the effects of this coating from sediment metals, aqueous metals, and pH using a multiple regression analysis.

Long-term assessment of the biological integrity of streams in the Leviathan Mine watershed will require continuation of a monitoring program to ensure data are available to inform adaptive management. Sampling in both spring and fall produces information on seasonal and demographic shifts, revealing natural patterns in community and population ecology as well as problems arising from incomplete control of mine pollutants at different times. Monitoring at Aspen and Leviathan below the mine will provide a measure of the most difficult conditions for recovery nearest the source areas of contamination, while survey of Leviathan and Bryant above and below Mountaineer provides ongoing feedback on the success of treatment activities in ultimately restoring ecological integrity. Sampling at Mountaineer and other control stations, some external to the Leviathan watershed, will continue to be useful in framing the target range for attaining the desired condition of unimpaired community composition.

#### Conclusions:

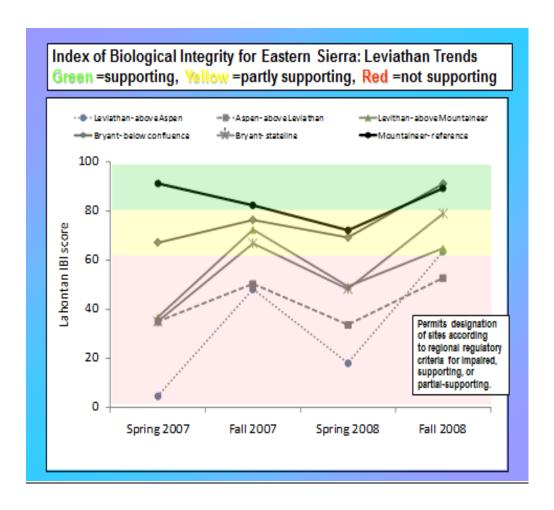
Bioassessment monitoring in the Leviathan Mine watershed has shown varied responses in biological integrity on sites exposed to AMD through 2009. After initial improvements, surveys performed in spring and fall of 2005 and 2006 showed that the populations at Leviathan Creek sites and Aspen Creek lost richness and density, and comprised more pollution-tolerant types of taxa. These changes correspond to the uninterrupted discharge of Delta Seep to Leviathan Creek during 2004-06. In contrast, recent data attest to improved conditions across most sites, approaching reference stream metrics. The instability of community structure and tolerance measures over time at many of the AMD-affected sites attests to their being in a state of shifting composition and functionality as exposure to chemical pollution fluctuates.

The following recommendations are based on monitoring data to date:

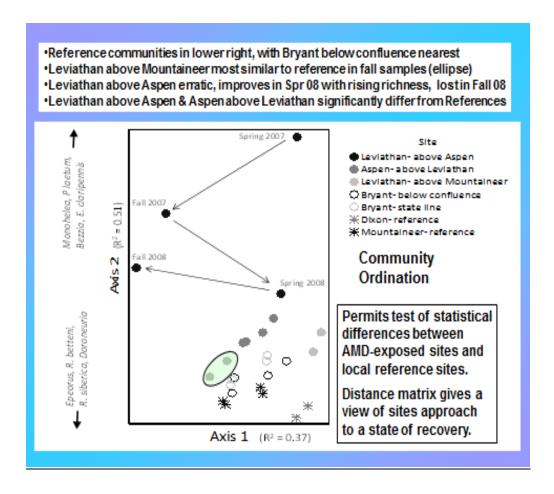
- 1. In order to interpret how different remediation activities are related to changes in the stream communities of the Leviathan Mine drainage, the biological response patterns should be coupled to a chronology of the timing, locations, and types of operations that have affected the volume and quality of treated flow. This discharge information, along with water chemistry data, will permit evaluation of the effectiveness of individual and cumulative treatments, and correlation of chemical improvements in water and sediment with ecological recovery.
- 2. Further analysis of the complete bioassessment dataset to include (1) community ordination of taxonomic similarity (such as non-metric multidimensional scaling) to graphically distinguish over time how changes in the invertebrate fauna of AMD-exposed sites compare to the fauna of local and external control sites and are related to metal contaminants of water and sediments, (2) calculation of a multimetric Index of Biological Integrity (IBI) based on that developed for the Lahontan Region (Herbst and Silldorff, 2009) that uses combined metrics scaled relative to reference streams from throughout the eastern Sierra [refer to graphs on p.16-17 as examples of analysis approaches 1 and 2], and (3) comparisons of the food web dynamics of the stream through partitioning of the functional feeding group composition of the invertebrate communities.

# Further Analysis Examples:

Example of application of Index of Biotic Integrity to Leviathan data for the years 2007-2008.



Example of Ordination Analysis for interpreting community changes over time and approach to reference condition (2007-2008 data only):



#### Acknowledgements

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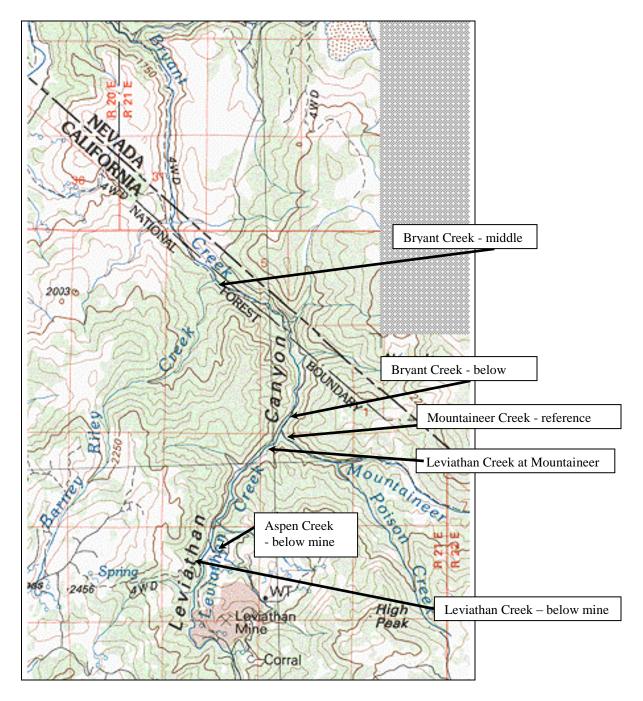


Figure 1. Locations of key sample sites surveyed for aquatic invertebrate biomonitoring of the Leviathan Mine watershed.

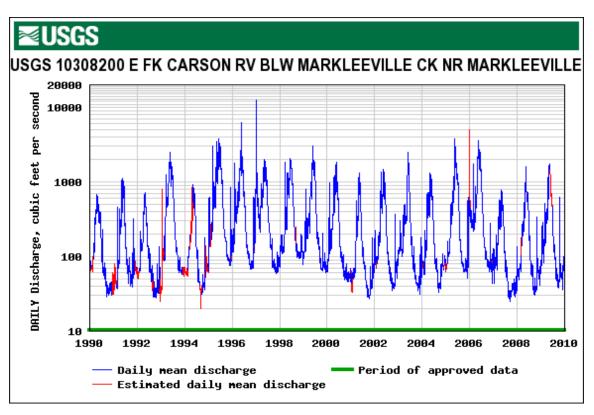


Figure 2. USGS hydrograph for E Fork Carson River (downriver of Bryant) 1990-2010.

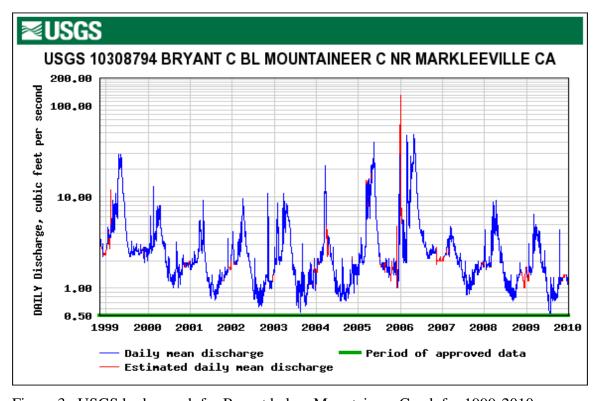


Figure 3. USGS hydrograph for Bryant below Mountaineer Creek for 1999-2010.

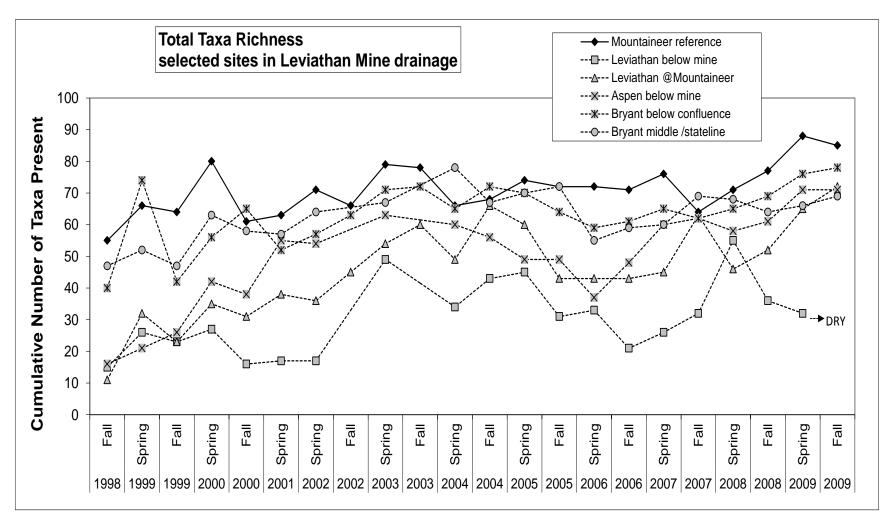


Figure 4. Richness expressed as the combined number of total taxa present from 5 samples at each site over time (season and year) for selected sites in the Leviathan Mine watershed. Solid line for reference site, dashed lines for AMD-exposed sites.

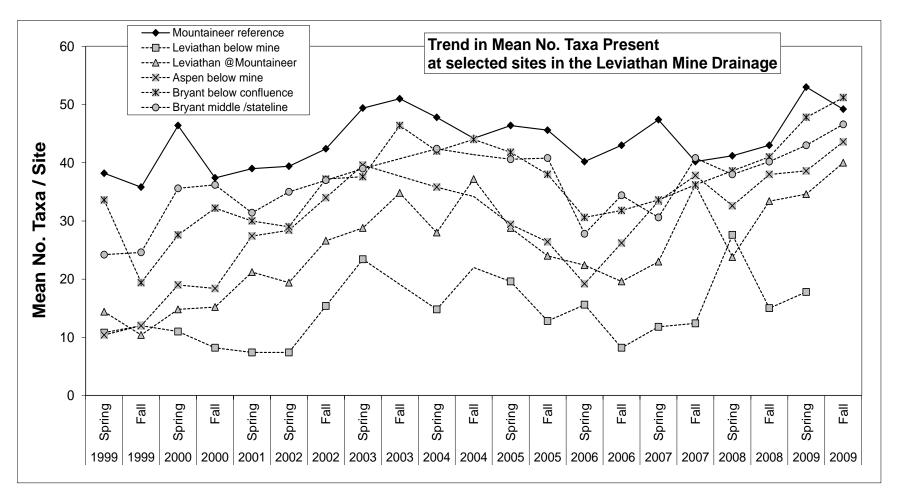


Figure 5. Richness expressed as mean number of taxa present in the 5 replicate samples at each site over time (season and year) for selected sites in the Leviathan Mine watershed. Solid line for reference site, dashed lines for AMD-exposed sites.

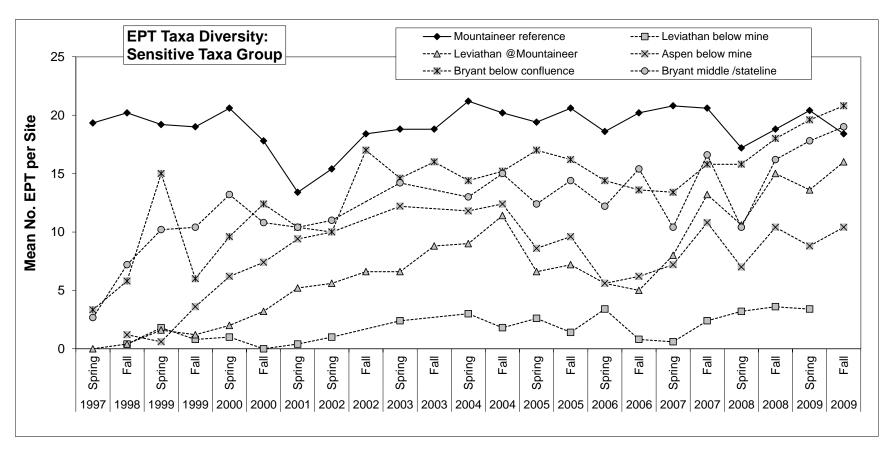


Figure 6. Richness expressed as mean number of EPT taxa present in the 5 replicate samples at each site over time (season and year) for selected sites in the Leviathan Mine watershed. Solid line for reference site, dashed lines for AMD-exposed sites.

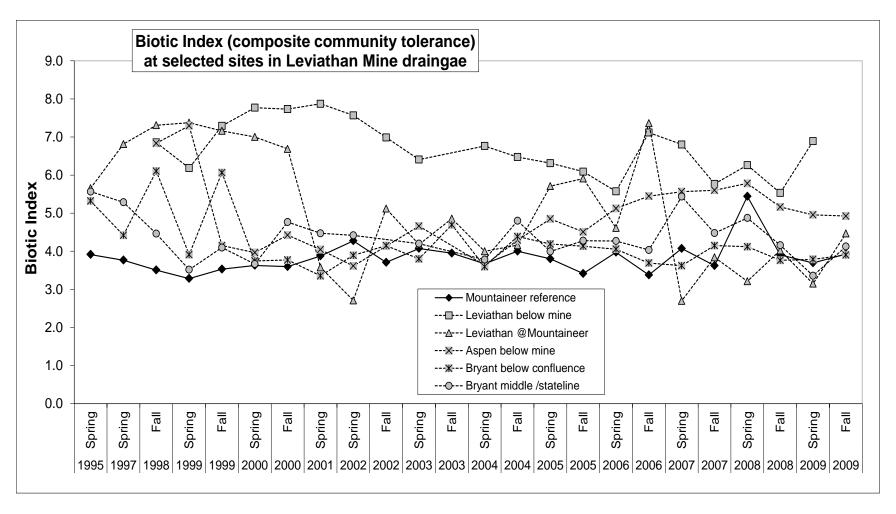


Figure 7. Biotic Index as the mean of 5 replicate samples at each site over time (season and year) for selected sites in the Leviathan Mine watershed. Solid line for reference site, dashed lines for AMD-exposed sites.

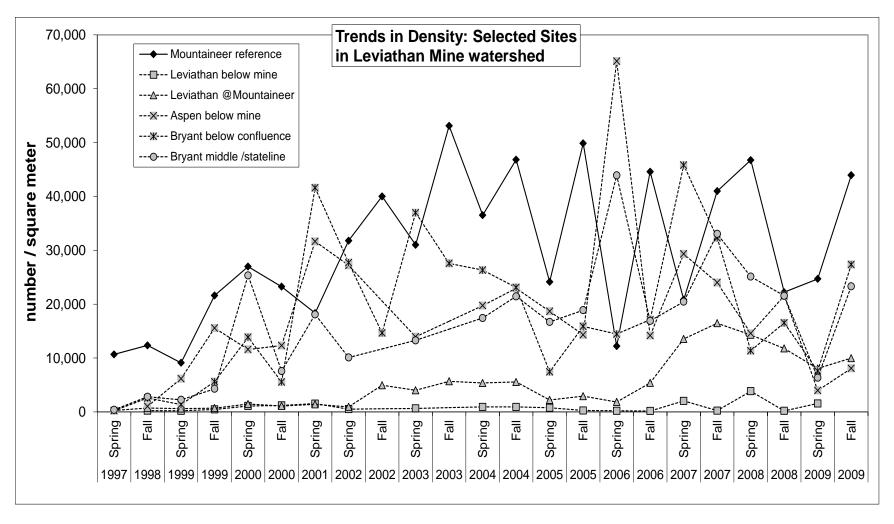


Figure 8. Average density (number / square meter) of total invertebrates from 5 replicate samples at each site over time (season and year) for selected sites in the Leviathan Mine watershed. Solid line for reference site, dashed lines for AMD-exposed sites.

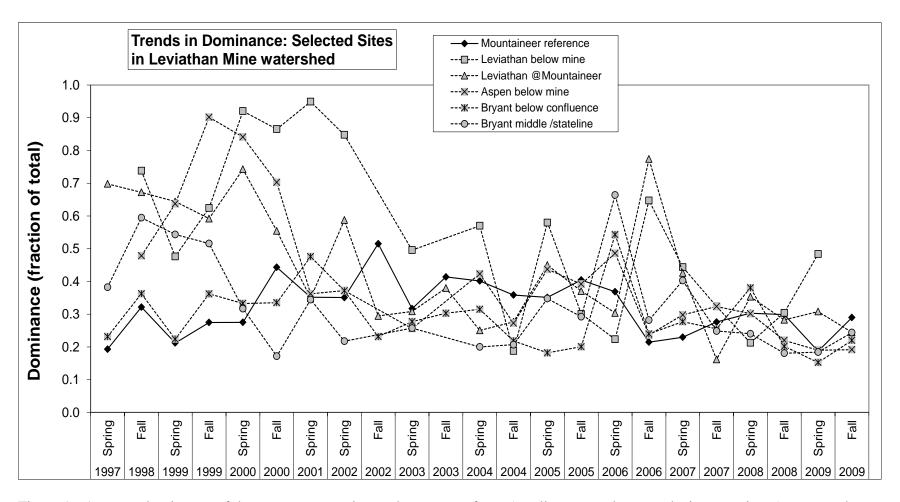


Figure 9. Average dominance of the most common invertebrate taxon from 5 replicate samples at each site over time (season and year) for selected sites in the Leviathan Mine watershed. Solid line for reference site, dashed lines for AMD-exposed sites.